Kidney International, Vol. 41 (1992), pp. 966–972

Renal hypertrophy in streptozotocin diabetic rats: Role of proteolytic lysosomal enzymes

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Renal hypertrophy in streptozotocin diabetic rats: Role of proteolytic lysosomal enzymes. Renal protein mass increases in diabetic renal hypertrophy. Accretion of protein may be the result of increased protein synthesis and/or decreased protein degradation. The lysosomal proteases, cathepsins B and L, are key enzymes in cellular protein catabolism. To evaluate the role of protein degradation in diabetic renal hypertrophy, the activities of cathepsins B and L were measured in microdissected proximal tubule segments and in kidney cortex homogenates. In rats four and ten days following induction of diabetes by streptozotocin, the kidney weight was increased and the cathepsin activities were reduced in proximal tubule segments. Treatment with insulin prevented both changes. The liver weight in diabetic rats was decreased and the activity of cathepsins B and L was increased, while the activity in kidney cortex was reduced. This excluded that diabetes *per se* may be accompanied by decreased cathepsin activities independent of organ hypertrophy. Renal hypertrophy as a cause rather than as the consequence of reduced cathepsin activities was excluded by the finding of unchanged cathepsin activities in proximal tubule segments from rats with compensatory renal hypertrophy four days and ten days following unilateral nephrectomy. Decreased activities of cathepsins B and L may reflect decreased intracellular protein degradation. Decreased protein breakdown in proximal tubules may contribute to diabetic renal hypertrophy. In agreement with this interpretation are the results from rats six months following induction of diabetes. Renal hypertrophy is complete at that time. No further accretion of protein occurs and the cathepsin activities in the proximal tubule were not different from controls.

The kidney size is increased in the initial phase of human type I diabetes and in early experimental diabetes in rats [1, 2]. Diabetic renal hypertrophy is associated with an increase in renal protein mass [2–5]. Accretion of protein may be the result of increased protein synthesis and/or decreased protein catabolism. Protein synthesis as well as protein degradation are energy dependent processes [6]. Hence, decreased cell protein breakdown would be an energy saving mechanism to increase kidney protein mass. The lysosomes are generally believed to play a major role in the breakdown of proteins in mammalian cells [6–10]. Protein degradation by the lysosomal system involves sequestration of intracellular proteins in autophagic vacuoles, fusion of these vacuoles with primary lysosomes, and degradation of proteins within the newly formed secondary lysosomes by proteinases. Highly active lysosomal proteinases are the cathepsins B and L. Inhibition of cathepsins B and L inhibits intracellular protein degradation by up to 70% [8, 9]. To evaluate the role of lysosomal proteolysis in diabetic renal hypertrophy the activities of cathepsins B and L were measured in kidneys from rats with streptozotocin-induced diabetes, from control rats, and from streptozotocin-injected rats treated with insulin. Renal hypertrophy in streptozotocin-injected rats was prevented by insulin treatment. To evaluate the possibility that hypertrophy *per se* may be accompanied by changes in activity of cathepsins B and L, the enzymes were determined in hypertrophic kidneys from rats following unilateral nephrectomy. Since renal hypertrophy involves mainly kidney cortex and proximal tubule is the major component of kidney cortex [2], enzyme activities were determined in kidney cortex homogenates and in microdissected segments of the proximal tubule. For comparison, the activity of cathepsins B and L was also measured in liver homogenates from diabetic and nondiabetic rats.

Received for publication March 27, 1991
and in revised form October 1, 1991
Accepted for publication October 3, 1991

Methods

Experimental animals

Eight- to ten-week-old female Sprague-Dawley rats weighing 180 to 250 g were studied (Central Animal Laboratory, Medical School Hannover, Germany). The animals were housed in metabolic cages and had free access to standard rat chow (Altromin, Spezialfutter GmbH, Lage, Germany) and tap water.

Experimental protocol

Diabetes was induced by intravenous injection of streptozotocin (60 mg/kg body wt), in 1 ml of 0.1 mol/liter citrate buffer, pH 5; (Sigma Chemical Company, St. Louis, Missouri, USA). Rats with urine glucose lower than 3 g/24 hr were not included. Controls received 1 ml of citrate buffer only. Nine experimental groups were studied. Groups 1 to 6 consisted of rats four days, ten days, and six months following induction of diabetes and their respective controls. Group 7 was comprised of rats ten days following injection of streptozotocin treated with s.c. insulin, 2 × 4 units per day, beginning at day two. Depot insulin was used (Hoechst AG, Hoechst, Germany) which contains aminochinuridedihydrochloride (ACDC) as depot body and methyl-4-hydroxybenzoate (MHB) as the preservative. To evaluate possible effects of both substances on enzyme activities, a group of ten day control rats was injected with ACDC and MHB

in a dose and time schedule equivalent to insulin injection. Groups 8, 9, 10, and 11 consisted of rats four days following unilateral nephrectomy, rats ten days following unilateral nephrectomy, and rats four and ten days following sham operation. In all groups, kidney weight, creatinine clearance, urine protein, and activities of cathepsins B and L in proximal tubule segments were measured. In separate animals of the three groups ten days following induction of diabetes—controls, diabetics, diabetics with insulin—right kidney water content, left kidney protein content and activities of cathepsins B and L in kidney cortex homogenates were determined. In ten day diabetic rats and in controls, liver weight, liver protein and liver activities of cathepsins B and L were measured for comparison. Twenty-four hours prior to the experiments urine was collected and creatinine clearance and glucosuria were determined.

Determination of creatinine, protein, glucose, and kidney water content

Creatinine in urine and plasma was determined using the picric acid method (Beckman Creatinine Analyzer 2, Fullerton, California, USA). Protein in urine, kidney and liver homogenates was determined by Lowry's method using bovine albumin as standard [11]. Glucose in urine and blood was determined by the hexokinase/G6P-DH-method (Gluco-quant, Boehringer Mannheim GmbH, Mannheim, Germany). The dry weight of the right kidney was determined after four hours of desiccation in an oven at 125°C. The difference between wet and dry weight was assumed to be the water content of the kidney.

Preparation of homogenates from kidney cortex and liver

The animals were killed by stunning and cervical dislocation. Blood was withdrawn by cardiac puncture. Both kidneys and the liver were removed rapidly and immersed in ice-cold dissection solution. Previously weighed samples of kidney cortex (0.4 to 0.6 g) and liver (0.5 to 1.1 g) were minced and repeatedly frozen in dry ice and thawed. The samples were homogenized in 20 ml of a 10 mmolar phosphate buffer, pH 7.4, using a cooled Sorvall Omni-Mixer four times for four minutes at 50,000 RPM. Kidney cortex homogenate was further diluted 1:100 for cathepsins B and L determination and 1:30 for protein determination. Liver homogenate was diluted 1:50 for cathepsin and protein determinations.

Determination of cathepsins B and L in isolated segments of proximal tubules and in homogenates from kidney cortex and liver

The combined activities of cathepsins B and L in isolated tubule segments was measured by means of a fluorometric microassay as published recently [11]. Z-phenylalanyl-arginine-7-amido-4-methylcoumarin (Z-Phen-Arg-NMec) was used as substrate for cathepsins B and L together. The enzymes cleave the substrate and release 7-amino-4-methylcoumarin (NMec), which is highly fluorescent and can be measured at very low concentrations in small fluid volumes. The substrate is specific for cathepsins B and L [11].

Solutions

All solutions were prepared from glass distilled water to reduce background fluorescence. All chemicals were reagent grade. The dissection solution contained (in mmol/liter): NaCl, 136; KCl, 3; $MgSO_4$, 1.2; K_2HPO_4, 1; $CaCl_2$, 2; sodium lactate, 4; sodium citrate, 1; L-alanine, 6; and glucose 5.5. The collagenase solution was identical to the dissection solution except for the addition of 0.1% collagenase (Sigma, Type I). The rinsing solution contained (in mmol/liter): Na_2HPO_4, 11.5; KH_2PO_4, 55.2; and EDTA, 4. The pH was 6.0 and the osmolality was 141 mOsm/kg H_2O. The preincubation solution was the same as the rinsing solution but, in addition, contained 0.2% Triton X100 (Sigma) and 0.05% bovine serum albumin (Sigma). The substrate, Z-Phen-Arg-NMec, was obtained from Bachem Feinchemikalien, Bubendorf, Switzerland. A 10 mmolar stock solution of the substrate was prepared in DMSO (dimethyl sulphoxide). This stock solution was diluted daily to a 1 mmolar solution with 0.1% Brij solution (Sigma). To this final substrate solution, 8 mmol/liter cysteine were added immediately before use. The stop solution contained 100 mmol/liter iodoacetate in a buffer containing 30 mmol/liter sodium acetate and 70 mmol/liter acetic acid. The pH was 4.75. For preparation of standard curves, the reaction product NMec was obtained from Bachem Feinchemikalien, Bubendorf, Switzerland.

Microdissection of tubule segments

For the determination of cathepsins B and L in isolated segments of proximal tubules, the left kidney was removed rapidly and perfused with 30 ml of ice-cold collagenase solution to facilitate microdissection. The kidney was sliced along the cortico-medullary axis, and the slices were incubated in collagenase solution gassed with 100% oxygen in a shaking water bath at 37°C. Collagenase was used to facilitate dissection. The segments S1, S2, and S3 of the proximal tubule were microdissected from the slices in dissection solution as described previously [12]. In the present study S1 was identified as the first mm of the proximal tubule attached to the glomerulus, while S2 included the last mm of the proximal convoluted tubule (S2c) and the first mm of the pars recta (S2r). S3 was identified as the last mm of the pars recta immediately prior to the thin descending loop of Henle. The dissected segments were straightened and the length was measured using an eyepiece micrometer. The length varied from 300 to 1000 μm. Each segment was then transferred into a second dissection dish with rinsing solution. After 10 seconds the segments were transferred with a 2 μl volumetric pipette into a reaction vial containing 25 μl of preincubation solution. In each rat, between three and seven samples of each segment were dissected and analyzed.

For the determination of cathepsins B and L in homogenates of kidney cortex and liver, 2 μl of the diluted homogenates were added to 25 μl of preincubation solution contained in the reaction vial, and the following steps were identical for isolated tubule segments and homogenates. The vials were sealed and frozen in dry ice for ten minutes before preincubation was initiated. Homogenates were analyzed in duplicates.

Incubation

The samples were preincubated for ten minutes in a shaking water bath at 37°C. The preincubation in the hypoosmolar preincubation solution lysed the tubules and tissues completely. Next, the vials were kept on ice while 25 μl of substrate solution were added. At this point the samples were incubated for 30 minutes in a shaking water bath at 37°C. The enzyme reaction

Table 1. Functional parameters of rats four days, ten days, and six months following induction of diabetes by streptozotocin injection, and of rats four and ten days following unilateral nephrectomy (Nx)

Group	N	Body wt *g*	Kidney wet wt *mg*	Glucose serum *mg/100 ml*	Glucose urine *mg/24 hr*	Urine volume *ml/24 hr*	Creatinine clearance *ml/min/100 g body wt*	Urine protein *mg/24 hr*
4 Days								
Control	8	238 ± 7	812 ± 82[a]	133 ± 18[a]	38 ± 14[a]	13 ± 2[a]	0.74 ± 0.2	5 ± 2
Diabetes	10	227 ± 16	947 ± 39	502 ± 63	7093 ± 1958	75 ± 31	0.65 ± 0.13	7 ± 2
Sham operated	6	232 ± 12	903 ± 35	nm	nm	18 ± 3	0.81 ± 0.24	6 ± 2
Unilateral Nx	6	220 ± 9	1036 ± 83[b]	nm	nm	17 ± 4	0.52 ± 0.04[b]	5 ± 1
10 Days								
Control	15	237 ± 6[a]	790 ± 81[a]	127 ± 15[a]	18 ± 8[a]	14 ± 5[a]	0.48 ± 0.18[a]	6 ± 2[a]
Diabetes	14	219 ± 16	936 ± 81	417 ± 106	9570 ± 3750	97 ± 29	0.65 ± 0.21	16 ± 6
Diabetes + insulin	10	231 ± 12[a]	847 ± 67[a]	133 ± 50[a]	1132 ± 580[a]	27 ± 11[a]	0.44 ± 0.20[a]	8 ± 4[a]
Sham operated	7	220 ± 13	870 ± 30	nm	nm	20 ± 4	0.76 ± 0.22	66 ± 2
Unilateral Nx	7	217 ± 15	1080 ± 90[b]	nm	nm	14 ± 6[b]	0.48 ± 0.12[b]	5 ± 1
6 Months								
Control	9	289 ± 26[a]	970 ± 130[a]	127 ± 15[a]	21 ± 8[a]	12 ± 4[a]	0.57 ± 0.07[a]	11 ± 20[a]
Diabetes	10	238 ± 25	1480 ± 220	479 ± 62	5220 ± 920	95 ± 48	0.98 ± 0.32	94 ± 58

Values are mean ± SD. Abbreviation is nm, not measured
[a] $P < 0.05$ versus the respective diabetes group
[b] $P < 0.05$ versus unilateral nephrectomy

was stopped by placing the vials on ice and adding 500 μl of stop solution. To evaluate the background fluorescence due to either substrate contamination with reaction product or spontaneous hydrolysis of the substrate during incubation, vials containing 25 μl of preincubation solution and 25 μl of substrate solution without nephron segments or tissue homogenate were treated identically. A standard curve was measured with each experiment.

Measurement of fluorescence

The fluorescence was determined in microcuvettes using a Turner Model III fluorometer (Turner Assoc., Palo Alto, California, USA). The sample was excited at 365 nm (Corning filter CS-7-83), and the emission was recorded at 450 nm (Corning filter CS-3-72). The enzyme activity in proximal tubule segments was expressed in pmol of released reaction product NMec per mm tubule length per minute of incubation time. Activities of cathepsins B and L in kidney cortex and liver were expressed in nmol released reaction product per mg protein per minute of incubation time. The assay was linear with respect to incubation time, tubule length, and weight of tissue [11].

Statistics

Values are given as mean ± SD. The significance of differences was tested by Student's *t*-test. For multiple comparisons between controls, diabetes, and diabetes + insulin the one-way analysis of variance (ANOVA) was used. Where ANOVA was significant, Duncan's multiple-range test was used to identify group differences. Differences in proteinuria were evaluated by Wilcoxon's rank sum test. P values < 0.05 were considered significant.

Results

Effects of ACDC and MHB

Seven non-diabetic rats of the ten day group received daily injections of depot body (ACDC) and preservative (MHB). This treatment did not change the values of body weight, kidney weight, urine volume, creatinine clearance, proteinuria, plasma glucose, and urine glucose in comparison to control animals ($N = 8$). Activities of cathepsins B and L in proximal tubule segments remained unchanged. Hence, both groups were taken together as control group for the following experiments.

Effects of four days diabetes

Rats four days following streptozotocin injection had increased values of serum glucose, glucosuria, and urine volume (Table 1). The kidney weight was increased by 17% in comparison to controls ($P < 0.01$). The activities of cathepsins B and L were decreased in the proximal tubule segments S2c, S2r, and S3, ($P < 0.05$ vs. control, respectively) (Fig. 1).

Effects of 10 days of diabetes and insulin treatment

Ten days following streptozotocin injection increased values of blood glucose, urinary glucose, and urine volume were present (Table 1). Kidney wet weight was increased by 19% to 27% ($P < 0.001$), kidney protein content was increased by 20% ($P < 0.03$), and kidney water content remained unchanged at 75% (Tables 1 and 2). The body weight was lower in diabetic rats ($P < 0.05$ vs. control), the creatinine clearance was elevated ($P < 0.05$ vs. control) and proteinuria was 250% of control values ($P < 0.001$). Injection of insulin prevented most of the changes observed in diabetic rats (Tables 1 and 2). Body weight, kidney weight, kidney water content, kidney protein, creatinine clearance, urine protein, and serum glucose were not different from controls. Urine glucose and urine volume were elevated in the insulin group ($P < 0.001$ vs. control, respectively). In comparison to diabetic rats, the insulin treated animals had lower values of kidney weight ($P < 0.05$), kidney protein ($P < 0.05$), urine volume, urine protein excretion, creatinine clearance, blood glucose, and urine glucose (Tables 1 and 2). The activities of cathepsins B and L from diabetic rats were decreased in kidney cortex homogenates ($P < 0.05$ vs. control) (Table 2), in S1 segments ($P < 0.01$) and in both S2 segments ($P < 0.001$, respectively; Fig. 2). Insulin treatment prevented the decrease in activity of cathepsins B and L in the kidney cortex of streptozotocin injected rats (Table 2). The enzyme activities

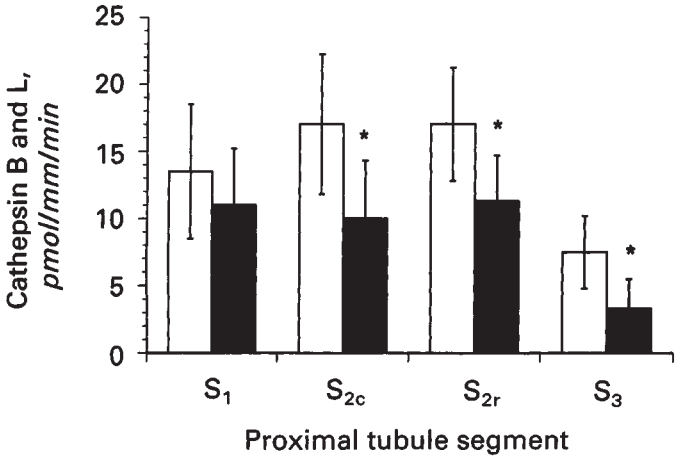

Fig. 1. *Activities of cathepsins B and L in microdissected proximal tubule segments from rats four days following injection of streptozotocin and controls.* S1, the first mm of the proximal tubule attached to the glomerulus. S2c, the last mm of the proximal convoluted tubule. S2r, the first mm of the pars recta. S3, the last mm of the pars recta immediately prior to the thin descending loop of Henle. Open bars represent values from control rats ($N = 8$). Dark bars represent values from streptozotocin-diabetic rats ($N = 10$). Data are mean ± SD. The asterisks indicate $P < 0.05$ versus control.

in S1 and S2 segments from insulin treated rats were not different from activities in control rats. In comparison to diabetic rats, activities of cathepsins B and L were higher in S2r ($P < 0.005$) and in S2c ($P < 0.001$), but were not different in S1 and in S3 (Fig. 2).

The liver weight in diabetic rats was decreased by 29% and activity of cathepsins B and L was increased by 52% ($P < 0.001$, respectively; Table 2).

Effects of six month diabetes

The rats had increased values of blood glucose, urine glucose and urine volume (Table 1). Creatinine clearance and proteinuria were elevated. The kidney weight was increased by 53%. The body weight was lower than in control animals. The activities of cathepsins B and L in the three segments of the proximal tubule were not different in diabetic and control rats, as shown in Figure 3.

Effects of unilateral nephrectomy

Unilateral nephrectomy had no effect on body weight, proteinuria, and urine volume (Table 1). Kidney weight increased by 15% after four days ($P < 0.05$) and by 28% after ten days ($P < 0.001$). The creatinine clearance of the left kidney following contralateral nephrectomy was increased in comparison to 50% of the clearance of sham operated rats in both groups ($P < 0.005$, respectively). Activities of cathepsins B and L are depicted in Figure 4. No differences in cathepsin activities from four days and ten days in sham operated rats were present. Therefore, the values of both groups are presented together in one column. A significant increase in comparison to controls occurred in the S2r segment of the proximal tubule four days following unilateral nephrectomy ($P < 0.02$). The activities of cathepsins B and L in all other segments remained unchanged at four days as well as at ten days following unilateral nephrectomy.

Discussion

The kidney weight in streptozotocin diabetic rats was increased by 17% at day four, by 18% to 27% at day ten, and by 53% at six months following induction of diabetes in comparison to the respective controls. The kidney protein content at ten days was 20% above control values, and the water content of the kidneys was the same in diabetics and controls. This indicates accretion of protein mass in diabetic renal hypertrophy, as described previously [12]. Renal hypertrophy was accompanied by reduction in activities of cathepsins B and L in both S2 segments and in the S3 segment at four days, and by reduction in S1 and S2 segments at ten days following induction of diabetes. Streptozotocin-induced diabetes was mitigated by insulin treatment, and renal hypertrophy as well as the decrease in cathepsin activities were almost completely prevented by insulin.

The activities of cathepsins B and L were measured as marker enzymes for the proteolytic activity in the lysosomes. The assay is specific and sensitive, and the control values in proximal tubule segments were similar to previously reported data [11]. Cathepsin analysis in isolated tubule segments with relation of enzyme activity to tubule length was applied since the usual relation of enzyme activity to mg protein in tissue homogenates is difficult to interpret in the context of renal hypertrophy. A hypothetical kidney cell may contain 100 mg of protein and 100 units of cathepsin. The enzyme activity would be 1 U/mg. Reduction of the activity to 0.66 units per mg protein in the presence of cell hypertrophy could indicate: (1) reduced absolute activity, 80 U/120 mg protein; (2) unchanged absolute activity, 100 U/150 mg protein; and (3) increased absolute activity, 120 U/180 mg protein. Important for the role of cathepsins in relation to cell hypertrophy is the absolute enzyme activity per cell. Relation of enzyme activity to tubule length is a close approach to this optimal experimental design. However, determinations of cathepsin activities in renal cortex were performed in the present study to enable comparisons with liver cathepsin activities and with the results of other authors.

Factors that must be considered in the interpretation of the decreased cathepsin activities in diabetic animals include streptozotocin, the presence of proteinuria, renal hypertrophy *per se*, and diabetes mellitus. The prevention of the cathepsin reduction in kidneys of streptozotocin-injected rats by insulin excludes streptozotocin as a causal factor. Proteinuria stimulates cathepsin activity in the proximal tubule, and decreased enzyme activities may be related to reduced proteinuria [11]. This explanation appears unlikely since proteinuria in the diabetic rats was increased. Renal hypertrophy could be accompanied by a decrease in cathepsin activity. Proximal tubule length increases in diabetic and compensatory renal growth [2, 5], and enzyme activity was related to tubule length in the present study. Hence, it is conceivable that the true cathepsin activity remained unchanged, while the measured activity was decreased due to "dilution" in a longer tubule. This can be excluded since compensatory renal growth of similar magnitude and duration after unilateral nephrectomy was not accompanied by a decrease in cathepsin activities. Furthermore, the normal

Table 2. Body weight, kidney weight and water content, kidney protein, liver weight, and cathepsin B and L activities in kidney and liver homogenates of diabetic and non-diabetic rats

Group	N	Body wt g	Kidney wet wt mg	Kidney water content %	Kidney protein mg	Cathespins B and L kidney nmol/min/ mg protein	Liver wet wt g	Cathespins B and L liver nmol/min/ mg protein
10 Days								
Control	12	228 ± 13[a]	744 ± 51[a]	75.6 ± 2.1	87 ± 10[a]	174 ± 18[a]	8.6 ± 0.9[a]	25 ± 5.8[a]
Diabetes	10	209 ± 7	945 ± 93	74.8 ± 2.3	104 ± 11	131 ± 31	6.1 ± 0.3	38 ± 5.9
Diabetes + insulin	7	228 ± 5[a]	809 ± 47[a]	72.7 ± 2.4	81 ± 19[b]	223 ± 83[a]	nm	nm

Values are mean ± SD. Abbreviation is nm, not measured.
[a] $P < 0.05$ versus Diabetes

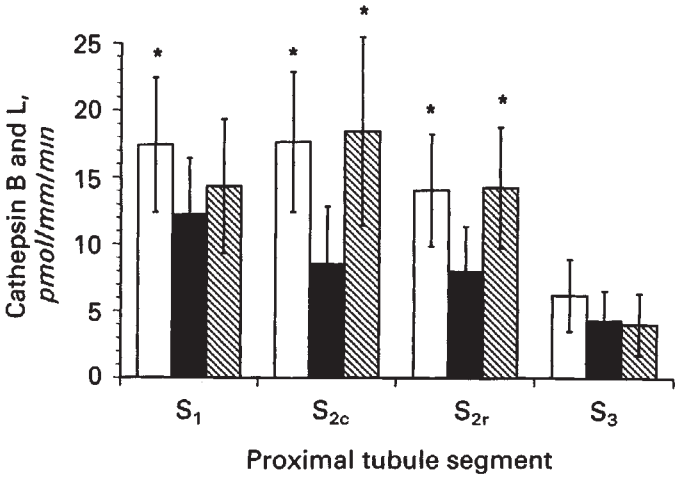

Fig. 2. *Activities of cathepsins B and L in microdissected proximal tubule segments from rats ten days following injection of streptozotocin and controls.* S1, the first mm of the proximal tubule attached to the glomerulus. S2c, the last mm of the proximal convoluted tubule. S2r, the first mm of the pars recta. S3, the last mm of the pars recta immediately prior to the thin descending loop of Henle. Open bars represent values from control rats ($N = 15$). Dark bars represent values from streptozotocin-diabetic rats ($N = 14$). Hatched bars represent values from streptozotocin-diabetic rats treated with insulin ($N = 10$). Data are mean ± SD. The asterisks indicate $P < 0.05$ versus untreated diabetic rats.

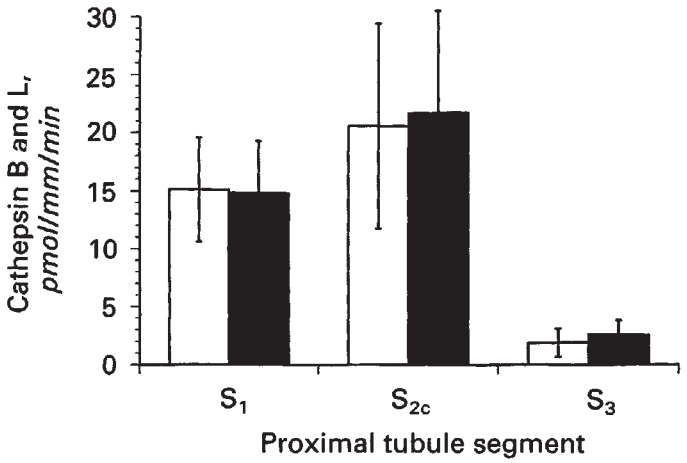

Fig. 3. *Activities of cathepsins B and L in microdissected proximal tubule segments from rats six months following injection of streptozotocin and controls.* S1, the first mm of the proximal tubule attached to the glomerulus. S2c, the last mm of the proximal convoluted tubule. S3, the last mm of the pars recta immediately prior to the thin descending loop of Henle. Open bars represent values from control rats ($N = 9$). Dark bars represent values from streptozotocin diabetic rats ($N = 10$). Data are mean ± SD.

cathepsin activities in proximal tubule segments from six-month-old diabetic rats in the presence of marked renal hypertrophy argue against renal hypertrophy as a causal factor for cathepsin reduction. Therefore, we believe that metabolic and/or functional changes related to the early diabetic state may be responsible for the decreased cathepsin activities in proximal tubule segments. Consistent with our results is the finding of reduced cathepsin D activity, another lysosomal proteinase, in kidney cortex homogenates from diabetic animals [13–15].

It is conceivable that the decreased cathepsin activities contribute to the development of diabetic renal hypertrophy for the following reasons. The cathepsin activities are reduced in the proximal tubule and diabetic renal hypertrophy involves proximal tubule [2]. A general decrease in cathepsin activities in early diabetes independent of organ hypertrophy was excluded by the finding of increased activities of cathepsins B and L in the liver and reduced liver weight. This is in agreement with data reported by others, showing a reduced protein content and increased rates of protein degradation in liver, muscle, and heart from diabetic rats [3, 14–19]. It should be mentioned, however, that some authors found increased activities of lysosomal proteases in muscle of diabetic animals [20], while others could not confirm these findings [21]. A decrease in cathepsin activities with a concomitant increase in protein mass is observed only in the kidney of diabetic rats. Furthermore, treatment of streptozotocin diabetic rats with insulin prevented renal hypertrophy and cathepsin decrease. Also consistent with the hypothesis of a causal relationship between reduced cathepsin activities and diabetic renal hypertrophy are the unchanged cathepsin activities in tubule segments from six-month-old diabetic rats. Renal hypertrophy is complete at that time. No further accretion of protein occurs [12], and proteolytic enzyme activities return to normal values.

The interpretation of the results depends on the critical question if reduced cathepsin activities reflect decreased cellular protein degradation. While we do not have direct proof that cathepsins B and L in the kidney indicate activities of cell protein degradation, several lines of evidence suggest a tight

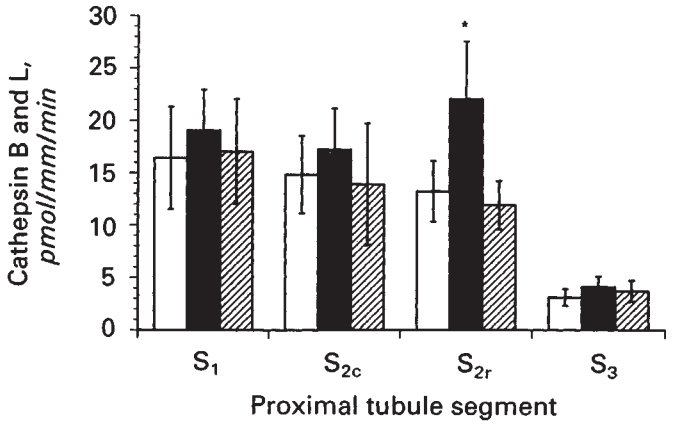

Fig. 4. *Activities of cathepsins B and L in microdissected proximal tubule segments from rats with compensatory renal hypertrophy four days and ten days following unilateral nephrectomy and from sham operated controls.* S1, the first mm of the proximal tubule attached to the glomerulus. S2c, the last mm of the proximal convoluted tubule. S2r, the first mm of the pars recta. S3, the last mm of the pars recta immediately prior to the thin descending loop of Henle. Open bars represent values from sham operated rats ($N = 13$). Dark bars represent values four days following unilateral nephrectomy ($N = 6$). Hatched bars represent values from rats ten days following unilateral nephrectomy ($N = 4$). Data are mean ± SD. The asterisks indicate $P < 0.05$ versus sham operated rats.

relationship. The inhibition of cathepsins reduced cellular protein degradation in other organs by up to 70% [9, 22, 23]. Increased protein degradation is associated with increased cathepsin activities [24–27], and the activities of cathepsin B in different organs were closely correlated with the rate of protein degradation in these tissues [28]. Finally, cathepsin B was decreased in regenerating liver tissue with decreased rates of protein degradation [29]. These data support the relation between activity of cathepsins B and L and the rate of intracellular protein degradation. In agreement with our hypothesis of decreased proteolysis in diabetic renal hypertrophy are in vivo measurements of protein turnover in kidneys from diabetic rats, which show an increased protein mass per kidney despite an unchanged rate of protein synthesis [3]. The decrease in cathepsin activities in S3 and S2 segments at four days and in S2 and S1 segments at ten days may therefore indicate that primarily S3 and S2 segments undergo hypertrophy, followed later by the S1 segment.

What may be the trigger for the decrease in cathepsin activities and the gain in protein mass in the kidney while other organs lose protein in the early diabetic state? The serum concentrations of branched chain amino acids (BCAA) including leucine, isoleucine and valine, were consistently found to be elevated in early diabetes [30–32]. BCCA are released predominately from the muscle of diabetics [30]. The increased serum concentration of BCCA certainly increase proximal tubule load and uptake [33] of these amino acids into proximal tubule cells where the concentration of the BCAA may increase [30, 34]. Since the BCCA leucine is a powerful inhibitor of protein degradation in muscle [35] and liver [36], its increased renal uptake may also inhibit protein degradation in proximal tubule cells and may contribute to diabetic renal hypertrophy. However, further experimental data are needed to substantiate this hypothesis.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft, Grant 0140/2-1. The technical help of Ch. Kruse is appreciated. Preliminary reports of the data were presented at the 20th Annual Meeting of the American Society of Nephrology, Washington D.C., December 13–16, 1987, and at the 19th Kongress der Gesellschaft für Nephrologie, Göttingen, 25.–28. September 1988.

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